

request that the present amendments and remarks be entered and be made of record in the file history of the present application.

CONCLUSION

No fee is believed due in connection with this submission. However, the Commissioner is authorized to charge any required fee or credit any overpayment to Pennie & Edmonds LLP Deposit Account No. 16-1150.

Respectfully submitted,

Date: October 26, 2001

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Enclosures

Exhibit A
Marked Up Versions of Replacement Paragraphs

On page 25, line 14, please replace the paragraph beginning, "STAT3 and STAT3 β are purified from baculovirus-infected Sf-9 insect cells with..." with the following paragraph:

STAT3 and STAT3 β are purified from baculovirus-infected Sf-9 insect cells with biotinylated M67SIE oligonucleotides. Briefly, Sf-9 cells are infected with baculoviruses encoding STAT3 or STAT3 β . 48 hours postinfection, cells are lysed with NP-40 lysis buffer (50 mM HEPES, pH 7.9, 150 mM NaCl, 1% NP-40, 20 mM NaF, 1 mM Na₃VO₄, 1 mM Na₄P₂O₇, 1 mM DTT, 0.5 mM PMSF, 2 mM EDTA, 0.1 μ M aprotinin, 1 μ M leupeptin, and 1 μ M antipain), and centrifuged (13,000 x g, 15 s, 4° C). The supernate cell lysates are supplemented with glycerol (to 10%) and 10 μ g of poly(dI-dC):poly(dI-dC) in a final volume of 1 ml, and incubated at 4° C for 30 min. Two micrograms of 5'-biotinylated DNA fragment, containing two copies of the M67SIE sequence (5'-AGCTTCATTCCCGTAAATCCCTA) (SEQ ID NO: 1) (Wagner, et al., 1990, EMBO J. 9:4477-4484), are then added and further incubated at 4° C for 2 hours with slow rotation. Subsequently, 100 μ l of avidin-agarose beads (50% slurry) is added to the mix and incubated for 30 min. The beads are then collected by centrifugation, washed 4X with NP-40 lysis buffer, and 3X with kinase buffer (25 mM HEPES; pH 7.5, 10 mM magnesium acetate). After final centrifugation (3,000 rpm, 2 min), the pellets of STAT3 and STAT3 β -bound Sepharose beads are incubated in 35 μ l kinase buffer containing approximately similar activities of purified p38 (AG Scientific), JNK (BIOMOL) or ERKs (BIOMOL) protein kinases for 5 min at room temperature. Subsequently, 5 μ l [γ -³²P]ATP solution (50 μ M ATP, 0.5 μ Ci/ μ l) is added and the mixture further incubated at 30° C. After 30 min, SDS-PAGE loading buffer was added, the samples are then electrophoresed on an SDS-8% polyacrylamide gel and exposed for autoradiography.

On page 35, line 13, please replace the paragraph beginning, "**Nuclear extracts and EMSA.** Nuclear extracts are prepared as previously described...." with the following paragraph:

Nuclear extracts and EMSA. Nuclear extracts are prepared as previously described. Briefly, nuclei are isolated and extracted in hypertonic buffer (20 mM HEPES, pH 7.9, 420 mM NaCl, 1 mM EDTA, 1 mM EGTA, 20% glycerol, 20 mM NaF, 1 mM Na₃VO₄, 1 mM Na₂P₄O₇, 1 mM DTT, 0.5 mM PMSF, 0.1 mM aprotinin, 1 mM leupeptin, and 1 mM antipain). Extracts are normalized for total protein, and 2-6 mg of protein is incubated with the ³²P-labeled high-affinity SIE probe, (5'-AGCTTCATTCCCGTAAATCCCTA-3') (SEQ ID NO: 2) derived from the *c-fos* gene promoter, as described (Yu, et al., 1995, Science 69:81-83; Garcia, et al., 1997, Cell Growth Diff. 8:1267-1276). Protein-DNA complexes are resolved on 5% non-denaturing polyacrylamide gels and analyzed by autoradiography. Controls are performed using rabbit polyclonal antibodies specific for STAT1, STAT3, or STAT5 proteins (Santa Cruz Biotechnology). The anti-Stat3 and anti-Stat5 antibodies supershift DNA-binding complexes, whereas the anti-Stat1 antibodies block complex formation (Yu, et al., 1995, Science 69:81-83; Garcia, et al., 1997, Cell Growth Diff. 8:1267-1276). For competition assays, nuclear extracts containing equal amounts of total protein are incubated with 100-fold molar excess of unlabeled SIE oligonucleotide or unlabeled irrelevant oligonucleotide, which contains the *c-fos* intragenic regulatory element (FIRE, 5'-GTCCCCCGGCCGGGGAGGCGCT-3') (SEQ ID NO: 3).

On page 37, line 1, please replace the paragraph beginning, "**RNA isolation and RT-PCR analysis.** Total RNA is isolated by lysis in ..." with the following paragraph:

RNA isolation and RT-PCR analysis. Total RNA is isolated by lysis in guanidine isothiocyanate followed by centrifugation through a cesium chloride gradient. cDNA is prepared from 200 ng of total RNA in a 40 ml reaction with AMV-RT (Boehringer-Mannheim). Specific gene amplification is performed on 5 ml of the cDNA reaction with the following primers: *bcl-2*: 5'-CGACGACTTCTCCGCCGCTACCGC-3' (SEQ ID NO: 4), and 5'-CCGCATGCTGGGCCGTACAGTCC-3' (SEQ ID NO: 5), which corresponds to bases 1761-1785; *bcl-x*: 5'-CGGGCATTCACTGACCTGAC-3' (SEQ ID NO: 6) and 5'-TCAGGAACCAGCGGTTGAAG-3' (SEQ ID NO: 7) which amplifies a 340 bp amplicon of *bcl-xL* or a 151 bp amplicon of *bcl-xS*; and *histone 3.3*: 5'-CCACTGAACCTCTGATTGCG-3' (SEQ ID NO: 8) and 5'-GCGTGCTAGCTGGATGTCTT-3' (SEQ ID NO: 9). Ten ml of

PCR products are electrophoresed on a 5% acrylamide gel and quantified by phosphorimaging using ImageQuant software (Molecular Dynamics).

On page 37, line 12, please replace the paragraph beginning, "Construction of plasmids. The murine *bcl-x* promoter reporter constructs ..." with the following paragraph:

Construction of plasmids. The murine *bcl-x* promoter reporter constructs are derived from a 3.2 kb genomic fragment containing the 5' region of the *bcl-x* gene upstream of the ATG translational start codon and have been described in detail (Grillot, et al., 1997, J. Immunol. 158:4750-4757). To construct the pGL2-mST1 reporter, three bases are mutated in the STAT1-binding motif (normal: TTCGGAGAA (SEQ ID NO: 10), mutant: TGAGGATAA (SEQ ID NO: 11) at position -315 to -307 (Grillot, et al., 1997, J. Immunol. 158:4750-4757) in the 600 bp fragment of the mouse *bcl-x* promoter. The equivalent site in the human promoter was mutated previously (Fujio, et al., 1997, J. Clin. Invest. 99:2898-2905). The pMvSrc vector encoding v-Src protein has been described (Turkson, et al., 1998, Mol. Cell. Biol. 18:2545-2552). To construct pIRES-Stat3b, the human STAT3b gene is excised from plasmid pSG5hStat3b by *Xba*I digestion, made blunt-ended with Klenow fragment of DNA polymerase, and subcloned into the *Eco*RV site of pIRES-EGFP vector (Clontech). The structure of pIRES-Stat3b is confirmed by restriction mapping, DNA sequencing, and functional analyses in transient transfections.

On page 63, please replace Table 1 with the following table:

TABLE 1. Peptides that Bind to Full-length STAT3 (12-mer Peptide Library).

Motif	Peptide Sequence	Frequency (out of 100 clones)
1	HY(S/P)PILVYQPSW (<u>SEQ ID NO: 12</u>)	25%
2	QDVHLTQQSRYT (<u>SEQ ID NO: 13</u>)	13%
3	SHPWNAQRELNV (<u>SEQ ID NO: 14</u>)	9%
4	YPAPQPLVTKTS (<u>SEQ ID NO: 15</u>)	8%
5	FSYPLTRAPLNM (<u>SEQ ID NO: 16</u>)	8%

On page 64, please replace Table 2 with the following table:

TABLE 2. Peptides that Bind the SH2 Domain of STAT3 (7-mer Peptide Library).

Motif	Peptide Sequence	Frequency (out of 100 clones)
1	HAIYPRN (SEQ ID NO: 17)	16%
2	ASTLPKA (SEQ ID NO: 18)	7%
3	IQSPHFF (SEQ ID NO: 19)	6%

On page 64, please replace Table 3 with the following table:

TABLE 3. Disruption of STAT3 DNA-binding Activity.

Peptide	IC ₅₀ (mM)
PY*LTKT (SEQ ID NO: 20)	280
PYLTKT (SEQ ID NO: 21)	ne
AY*LTKT (SEQ ID NO: 22)	204
PY*AKTK (SEQ ID NO: 23)	ne
PY*LATK (SEQ ID NO: 24)	289
PY*LKAK (SEQ ID NO: 25)	300
PY*LKTA (SEQ ID NO: 26)	320
PY*LK (SEQ ID NO: 27)	410
PY*FK (SEQ ID NO: 28)	1000
Y*LK (SEQ ID NO: 29)	ne
AY*LK (SEQ ID NO: 30)	365
Ac-Y*LK (SEQ ID NO: 31)	421
Ac-PY*LTKT (SEQ ID NO: 32)	212
PFLTKT (SEQ ID NO: 33)	ne
Ac-PY*LK (SEQ ID NO: 34)	156
PY*LA (SEQ ID NO: 35)	326
Ac-PY*LA (SEQ ID NO: 36)	288
PY*L (SEQ ID NO: 37)	182
AY*L (SEQ ID NO: 38)	147

(Y*=phosphotyrosine; ne=no effect; Ac=acetylation)

Exhibit B
Clean Versions of Replacement Paragraphs

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